Hypertension and Cardiovascular Risk Factors in Women

A follow-up study forty years after hypertensive pregnancies

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UNIVERSITY OF GOTHENBURG
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ABSTRACT

Hypertension and Cardiovascular Risk Factors in Women

A follow-up study forty years after hypertensive pregnancies

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The aims of the thesis were to investigate the impact of pregnancy blood pressure, a current diagnosis of hypertension and blood pressure levels on neurohumoral, cardiovascular and metabolic status in postmenopausal women and thus to explore possible contributing mechanisms to the increased cardiovascular risk following hypertensive pregnancies.

In this follow-up study after hypertensive- and normotensive pregnancies, 105 women were evaluated with the following methods: microneurography; office-, ambulatory- and central blood pressure measurements; anthropometric measurements; pulse wave velocity and augmentation index; carotid intima-media thickness; cardiovascular response to mental stress test and evaluation of perceived stress; echocardiography and laboratory analyses regarding metabolic and neurohumoral values. Another 160 women responded to a questionnaire regarding previous and present health.

Women with previous hypertensive pregnancies had an increased prevalence of a diagnosis of hypertension, increased pulse wave velocity and affected metabolic parameters compared to women with previous normotensive pregnancies. These findings may partly explain the increased cardiovascular risk following hypertensive pregnancies. The sympathetic activity was only increased in women with previous hypertensive pregnancies and present hypertension. High self-reported perceived stress was associated with increased waist circumference which, in turn is related to an increased cardiovascular risk. Higher blood pressure levels were related to early signs of left ventricular diastolic dysfunction, emphasizing the importance of rigorous blood pressure control.

Our study contributes with unique knowledge regarding women's health many years after hypertensive and normotensive pregnancies. A diagnosis of present hypertension seems to be of major importance for the increased cardiovascular risk after hypertensive pregnancies, why maintenance of normotension is essential for women with previous hypertension pregnancies in order to retain cardiovascular health after menopause.

Keywords: hypertension, pregnancy complications, follow-up studies, sympathetic nervous system, vascular stiffness, echocardiography, stress

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LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I Collén A-C, Manhem K, Sverrisdóttir YB. Sympathetic nerve activity in women 40 years after a hypertensive pregnancy.
J Hypertens 2012; 30:1203-1210

II Collén A-C, Hellgren M, Gustafsson H, Johansson M C, Manhem K. Cardiovascular and metabolic characteristics after hypertensive pregnancies.
J Hypertens 2013; 31:758-765

III Collén A-C, Gustafsson H, Hellgren M, Schiöler L, Bexander L, Manhem K. Impact of perceived stress on waist circumference in postmenopausal women.
Submitted

IV Collén A-C, Johansson M C, Wallentin Guron C, Gustafsson H, Manhem K. Echocardiographic changes in relation to blood pressure in postmenopausal women.
Submitted

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PAPER I-IV

ABBREVIATIONS

ABPM	Ambulatory blood pressure measurements
AIX	Augmentation index
Ao DBP	Central diastolic blood pressure
Ao SBP	Central systolic blood pressure
BI	Burst incidence
BF	Burst frequency
BMI	Body mass index
CWT	Stroop color word test
DBP	Diastolic blood pressure
DHEAS	Dehydroepiandosterone sulphate
HPA	Hypothalamic – pituitary – adrenal axis
HTP	Hypertensive pregnancies
LVMi	Left ventricular mass index
MSNA	Muscle sympathetic nerve activity
NTP	Normotensive pregnancies
PWV	Pulse wave velocity
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
WC	Waist circumference

INTRODUCTION

Five to ten percent of pregnancies are complicated by a hypertensive manifestation. Most women in industrialized countries experience a hypertensive pregnancy without major complications, yet hypertensive pregnancies remain a threat both to the woman and the fetus and are a major cause of maternal mortality worldwide[1]. Besides being hazardous during pregnancy and puerperium several epidemiological studies after hypertensive pregnancies have presented solid evidence of an increased maternal cardiovascular risk later in life[2-8].

Follow-up studies after hypertensive pregnancies are usually performed within months to a few years postpartum. Consequently studied women are often premenopausal and middle-aged, thus signs of affected cardiovascular or metabolic systems or clinical cardiovascular disease are rare. Furthermore, epidemiological results are based on registers why findings cannot give mechanistic explanations as to how the hypertensive pregnancy is linked to the increased cardiovascular risk. It seems however possible to find signs of affected structure and function in different target organs since hypertensive pregnancies can be severe conditions with long-term consequences, both for the mother and for the off-spring[9].

Whether it is the hypertensive pregnancy per se or the current blood pressure status and/or blood pressure levels that have the most impact on future health in women many years after hypertensive pregnancies is not much studied. The plausible association between blood pressure (previous and present) to factors connected to cardiovascular risk and disease is an interesting scientific field.

Preeclampsia or gestational (pregnancy induced) hypertension?

In a normal pregnancy, the cardiovascular system is affected by a number of changes[10]. Most pregnant women adapt to the increased load on the cardiovascular and metabolic systems without any further consequences, but some develop hypertensive (or metabolic) complications. Higher pre-pregnancy blood pressure levels, maternal overweight, heredity, age and metabolic deterioration such as elevated blood glucose and insulin resistance increase the risk to develop a hypertensive complication during pregnancy.

Despite a similar risk factor profile, the pathophysiological mechanisms behind preeclampsia and gestational hypertension differ. Gestational hypertension may be looked upon as a disposition for hypertension which is “revealed” when the cardiovascular system is encumbered during the pregnancy. The pathophysiological changes behind preeclampsia however, seem to start early after conception with defect trophoblastic invasion of the spiral arteries in the uterus. This causes decreased perfusion of the placenta, leading to excretion of vasoactive substances and activation of the immune system which affects the endothelial layer of the vasculature and other systems. Damaged endothelium is one of the mechanisms behind the subsequent rise in blood pressure levels, proteinuria and enhanced activity in the coagulation system[11,12].

In general, preeclampsia is considered a more severe condition than gestational hypertension. Also, the preeclamptic condition is usually graver the earlier signs or symptoms appear in the pregnancy. The consequences of preeclampsia can partly be treated during the pregnancy, but if the fetus is mature enough, delivery – i.e. removing the placenta - is the most adequate therapy.

Increased cardiovascular risk after hypertensive pregnancies

The pathways from hypertensive disorders of pregnancy to future cardiovascular disease are complex and multifactorial. A number of mechanisms are important in the pathological process, which all contribute solely and in interaction with one another. A few possible pathways linking hypertensive pregnancies and cardiovascular risk are discussed below, yet the mechanisms involved in the long-term consequences are far more complex and beyond the scope of this thesis.

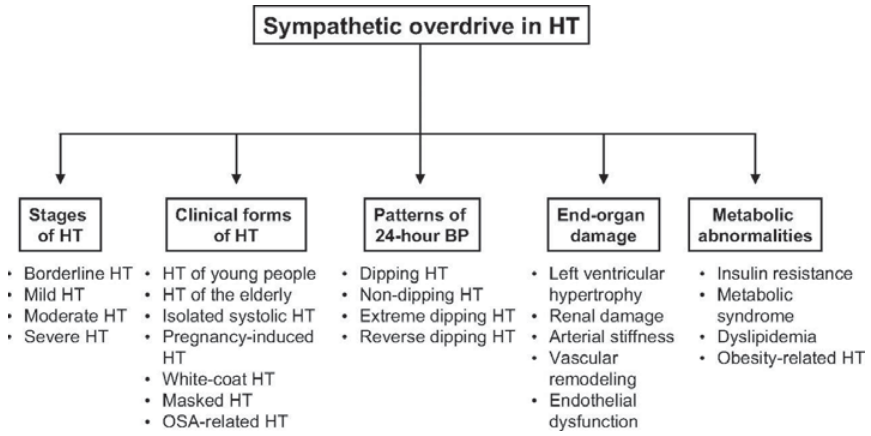
There are some common risk factors between hypertensive pregnancies in general and preclampsia in particular - and cardiovascular disease. These are (amongst others) elevated blood pressure, type II diabetes mellitus, lipid abnormalities, endothelial dysfunction and a disturbed fibrinolytic system[3,13,14]. Hypertensive pregnancies also exhibit features of metabolic abnormalities and enhanced sympathetic activity[15,16] which in turn are related to increased cardiovascular risk[17-20]. Alterations in these systems during hypertensive pregnancies might contribute to the increased cardiovascular risk shown in epidemiological studies.

More women with hypertensive pregnancies develop hypertension compared to women with normotensive pregnancies[21,22]. The strong correlation between hypertension and cardiovascular disease[23] makes the hypertension diagnosis per se a plausible explanatory mechanism behind the risk increase. Metabolic alterations are seen both in hypertensive pregnancies and in co-existence with hypertension[24], also increasing cardiovascular risk.

Another possible link between hypertensive pregnancies and later cardiovascular risk is increased sympathetic activity. It is well-known that sympathetic outflow is augmented during both normal and hypertensive pregnancies[20,25]. Besides enhanced activity during hypertension, increased sympathetic activity is present in a number of traditional cardiovascular risk factors, for example in visceral adiposity, diabetes mellitus and elevated blood lipids[26-28]. Hyperactivity of the sympathetic nervous system is a hallmark of cardiovascular manifestations[19,29], and hypertensive women seem to have a more pronounced autonomic dysfunction contributing to the elevated blood pressure when compared to age-matched men[30].

Since increased sympathetic activity has been shown in most forms of hypertensive manifestations (Figure 1), this again pinpoints that the diagnosis hypertension per se is of importance for cardiovascular morbidity.

Numerous studies have shown an age discrepancy between women and men regarding cardiovascular disease. In average, women are ten years older than men when they experience their first cardiovascular event[31]. Although the age difference partly can be



Grassi G. Hypertension, 2009. 54;4: 690-7.

Figure 1. Schematic drawing of the pathophysiological role of the sympathetic activation in hypertension, 24-hour blood pressure profile, end-organ damage, and metabolic abnormalities associated with a high blood pressure state.

explained by the more frequent occurrence of cardiovascular risk factors in younger men compared to age-matched women, the female sex hormones**,** in particular estrogen, are of importance for this delay in disease incidence. Estrogen has a complex impact on the cardiovascular system being protective in experimental models[32,33], but with contradictory results when used as a hormone replacement treatment after menopause[34].

Higher levels of serum testosterone have been shown in women with preeclampsia compared to women with normotensive pregnancies, both during pregnancy[35] as well as at follow-up[36] and it is speculated that this may contribute to cardiovascular risk. Estrogen levels do not seem to differ between hypertensive- and normotensive pregnancies to the same degree[37], although lower levels of estradiol in one study was a predictor for preeclampsia[38].

Arterial stiffness is a possible contributing factor connecting hypertensive pregnancies and the subsequent higher prevalence of hypertension and cardiovascular morbidity. Pulse wave velocity is a robust surrogate marker of arterial stiffness and increasing values correlate to higher risk for cardiovascular disease[39,40]. Increased pulse wave velocity after hypertensive pregnancies have been shown in small scale studies within a few years post-partum[41,42], but studies of the vasculature many years postpartum are rare - if any - and whether hypertensive pregnancies contribute to arterial stiffness is not known.

Hypertensive pregnancies and the heart

It is well established that hypertension is associated with left ventricular diastolic dysfunction[43,44] which, in turn, is associated with heart failure, cardiovascular diseases

and increased cardiovascular mortality[45-48]. Since hypertensive pregnancies increase the risk for future hypertension, examinations of the heart as a target organ for cardiovascular risk seem reasonable in these women. The few follow-up studies that have been performed with echocardiographic examinations after hypertensive pregnancies have shown contradictory results regarding persistent changes in the myocardial structure and function[49-51]. The longest of these studies was performed 13–18 years postpartum[52].

The pathological myocardial changes that are associated with hypertensive pregnancies seem to be similar to the early cardiac changes found in hypertension. These alterations include deterioration in left ventricular diastolic filling pattern[53] and signs indicating diastolic dysfunction, such as reduced longitudinal myocardial velocities and geometrical remodeling[54]. To further point to the association between hypertension, affected myocardium and cardiovascular morbidity, several studies have identified a correlation between diastolic dysfunction and measurements of vascular stiffness[55,56].

Cardiovascular disease and stress

In the last decades a link between psychological stress and clinical cardiovascular manifestations has been established[57]. Data from both epidemiological and prospective studies demonstrate associations between stress and myocardial infarction[57-60] as well as between stress and stroke[59,61]. Stressors, whether psychological or physical, activate the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis in order to create an adequate response to the stressor and to maintain homeostasis in the cardiovascular system[62,63]. Activation of the sympathetic nervous system leads to elevated blood pressure and heart rate inducing a “fight or flight” response, while activation of the HPA axis, in the short run, leads to increased levels of cortisol. Moreover, both the SNS and the HPA axis are involved in the physical outcomes related to psychological stress such as elevated ambulatory blood pressure levels[64], and an increased risk of coronary events[65].

A number of traditional cardiovascular risk factors, such as increased visceral adiposity, hypertension, raised levels of plasma glucose and blood lipids, exhibit enhanced activity in the SNS and disturbances in the HPA axis, hence connecting stress and metabolic disturbances[66]. Waist circumference can be used as a surrogate marker for visceral adiposity and is associated with cardiovascular risk and disease[67,68]. Studies investigating the possible relationship between stress and waist circumference are few and often with sparse or contradictory results[69,70].

In situations with acute stress (e.g. mental stress test) it is mainly the effects of the sympathetic nervous system that is responsible for the cardiovascular response. It has been shown that a greater reactivity to and poorer recovery from acute mental stress test predicts future cardiovascular risk[71].

AIM

The overall aims of the thesis were to investigate the impact of pregnancy blood pressure and a current diagnosis of hypertension and blood pressure levels on neurohumoral, cardiovascular and metabolic status in postmenopausal women and thus to explore possible contributing mechanisms to the increased cardiovascular risk following hypertensive pregnancies.

Specific objectives

1. Is the increased sympathetic nerve activity after hypertensive pregnancies persistent many years postpartum?

2. Do previous hypertensive pregnancies and/or present blood pressure status and levels influence:

    - Cardiac and vascular structure and function?
    - Metabolic and endocrine regulation?
    - Perceived and acute mental stress?

SUBJECTS AND METHODS

Ethics

The study was approved by the Ethics Committee at the University of Gothenburg and all participants gave oral and written consent before inclusion.

Study population

All subjects were recruited from the study population that comprised the material to the thesis *Hypertension in pregnancy* in which 261 women with a hypertensive manifestation during pregnancy and 260 women with normal pregnancies were included[72]. The 521 women gave birth at Sahlgrenska University Hospital/Östra during the years 1969–1973. Of the 261 women with a hypertensive manifestation during pregnancy, 164 women had preeclampsia and 97 women had gestational hypertension.

Since the investigation was performed about 40 years ago there is limited possibility to identify individual blood pressure measurement. However, Figure 2 shows blood pressures during pregnancy, after delivery and at a follow-up of women included in the study.

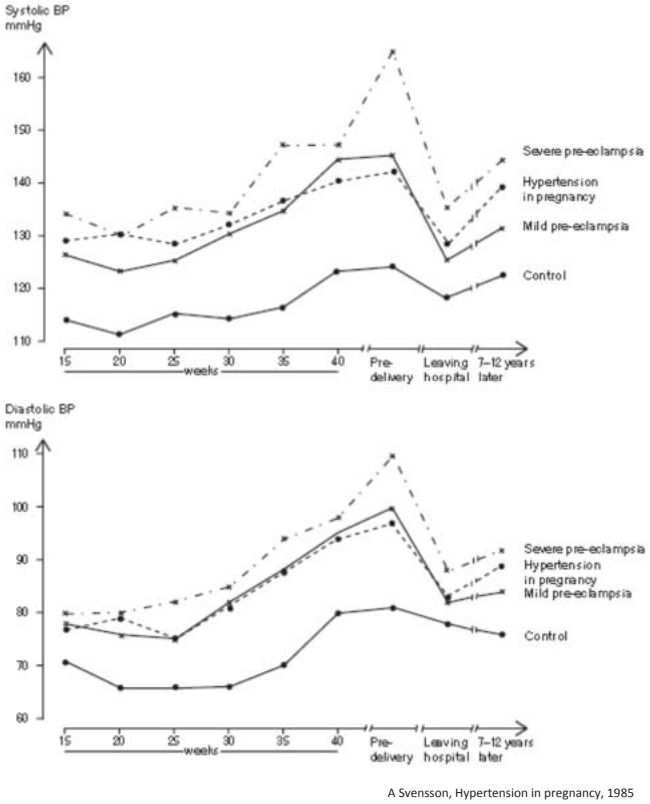
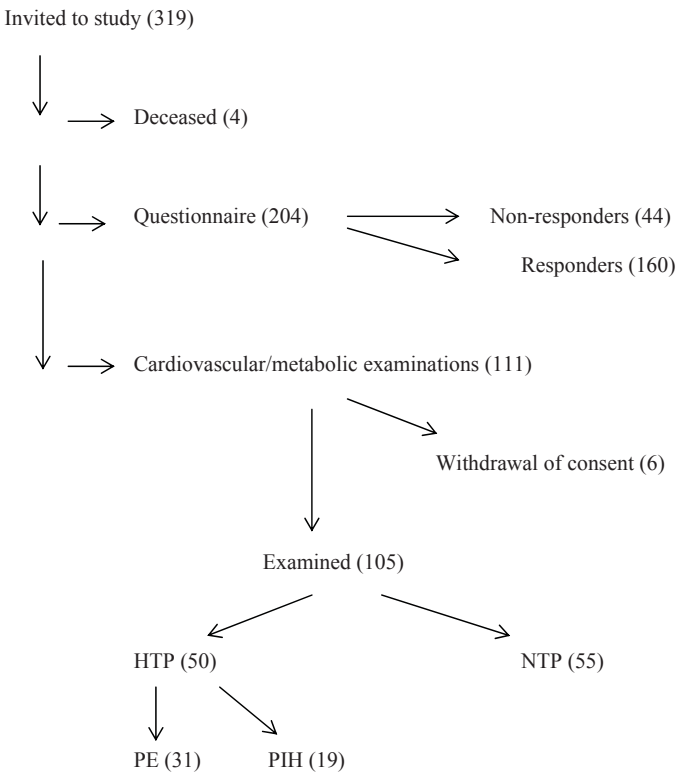


Figure 2. Mean systolic (upper panel) and diastolic (lower panel) blood pressures during pregnancy (15th-40th week of gestation) and after delivery, including a long-term follow-up.

To the studies included in this thesis, women from the original cohort living within 100 kilometers from Gothenburg were invited 35–40 years after pregnancy to a follow-up study. We were able to locate and invite 319 women, of whom 111 accepted the invitation (Figure 3*)*.

Of the 319 invited women, 208 did not participate in the clinical examinations. Four were deceased and the remaining 204 were followed-up by a questionnaire regarding their pregnancy during 1969–1973, as well as their present health status and medications.



HTP: hypertensive pregnancy, NTP: normotensive pregnancy, PE: preeclampsia, PIH: pregnancy induced hypertension. Figures within brackets denote number of women.

Figure 3. Flow-chart of study population.

Study design and settings

Papers I–IV are observational cross-sectional follow-up studies many years after hypertensive- and normotensive pregnancies.

The 319 women eligible for the study were invited randomly and irrespective of blood pressure status during previous pregnancy. The 105 women who participated in the clinical examinations were investigated during the years 2006–2010.

In Papers I–IV comparisons were made between women with hypertensive- and normotensive pregnancies respectively on different target variables. Apart from comparisons with respect to previous pregnancies, unique comparisons on different target variables were done in each paper; Paper I with main focus on sympathetic nerve activity; Paper II – cardiovascular and metabolic variables; Paper III – impact of perceived stress on cardiovascular and metabolic measurements and in Paper IV echocardiographic changes.

Paper I–IV

All women were examined at Sahlgrenska University Hospital/Östra regarding the clinical investigations, i.e. interviews, blood pressure measurements, carotid ultrasonography examinations, measurements of pulse wave and augmentation index and blood sampling. The interviews and regular clinical status of the subjects were performed by the author. All investigations were done by an experienced research nurse.

Paper I

The twenty-eight women who participated in the study regarding sympathetic activity were consecutively recruited from the 105 women participating to the follow-up study. All nerve recordings – as described below – were performed at the Department of Clincal Neurophysiology at Sahlgrenska University Hospital/Sahlgrenska by the same experienced examiner. The recordings were done under equal conditions and with the same equipment. The subjects' health records and health status when assessed were blinded to the examiner. The twenty-eight women were divided into three groups; group 1 consisting of eight women with previous hypertensive pregnancies and present hypertension. In group 2 ten women with previous hypertensive pregnancies and normotensive at study start were included. Group 3 consisted of ten women who represented controls. They had experienced normal pregnancies and were still normotensive when included in the study.

As a result of the consecutive inclusion no woman with a normotensive pregnancy and current hypertension was identified. This is in line with the fact that fewer women with normotensive pregnancies develop hypertension later in life compared to women with hypertensive pregnancies.

Paper I, II and IV

Echocardiographic examinations and measurements were performed at Department of Clinical Physiology at Sahlgrenska University Hospital/Östra. The same experienced echo technician did all echocardiographic investigations with the possibility to consult a specialist in Clinical Physiology when needed. The examiner was unaware of the individual study subject´s clinical data such as blood pressure levels and cardiovascular diagnosis.

Methods

Definitions

In 1969-1973 preeclampsia was defined as SBP ≥140 mmHg or DBP ≥90 mmHg and presence of coexisting proteinuria. Gestational (pregnancy induced hypertension)

was defined as SBP ≥140 mmHg or DBP ≥90 mmHg on more than one occasion. The definitions were in accordance with the Committee on Terminology of the American College of Obstetricians and Gynecologists[72]. Today´s definitions of preeclampsia and gestational hypertension have the same cut-off values for blood pressure, but are more clearly defined regarding proteinuria[73].

Gestational diabetes could not be identified as a separate diagnosis in ICD-8 which was the classification system used during 1969–1973. No woman in the study had type 1 diabetes mellitus during pregnancy since diabetic mothers were delivered at another hospital (Sahlgrenska Hospital) during this time period.

At follow-up in 2006-2010 study subjects were defined as having a diagnosis of hypertension, type 2 diabetes mellitus, myocardial infarction, angina pectoris, stroke or transitory ischemic attack based on their history. They were diagnosed a few to several years before entering the present study in accordance with current guidelines[74]. Women without a diagnosis of hypertension were categorized as normotensive. Many of the women with a current diagnosis of hypertension were well-controlled regarding their blood pressure levels with antihypertensive agents. Among women categorized as normotensive some had blood pressure levels above 140/90 mmHg when examined. If the blood pressure was persistently elevated when re-examined within a few days to a couple of weeks, they were referred to primary care for further controls and initiation of antihypertensive treatment when applicable.

All examined women in the study were caucasians. Smoking was categorized as no smoking or current smoking. Pregnancies were defined as hypertensive (preeclampsia or gestational hypertension) or normotensive from original data charts. Women with previous hypertensive pregnancies were defined as the HTP group and women with previous normotensive women as the NTP group.

Anthropometric measures

Body mass index (BMI) was calculated from weight in kilograms divided by squared value of height in meters. Waist circumference (WC) was measured in an up-right position midway between the lowest rib and the iliac crest with a non-stretchable tape. Measurements of weight and waist circumference were performed with the study subject in light underwear.

Blood pressure measurements

Office blood pressure and heart rate were measured in a sitting position after a ten minute rest with a validated automatic Omron 750IT (Omron Healthcare Co. Ltd, Kyoto, Japan) device. The size of the cuff was adjusted to the circumference of the subjects arm. One measurement was done in both arms followed by two more measurements in the arm with the highest values. Measurements were recorded at one to two minutes apart and blood pressure was reported as the mean of three readings.

Ambulatory blood pressure measurements (ABPM) was performed with SpaceLab ultralite ambulant blood pressure monitor 90217 (Spacelab Medical, Issaquah, WA, USA) in the non-dominant arm. The device was programmed to automatically measure blood pressure every 20 minutes during daytime (hours 06.00 a.m. –10.00 p.m.)

as well as during night time (hours 10.00 p.m.–06.00 a.m.). Mean values for systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated hourly for both awake and sleeping periods.

Microneurography

Direct recordings of multiunit efferent postganglionic muscle sympathetic nerve activity (MSNA) were obtained with a tungsten microelectrode with a tip diameter of a few microns, inserted into a muscle fascicle of the peroneal nerve posterior to the fibular head. A low impedance reference electrode was inserted subcutaneously a few centimeters away. When a muscle nerve fascicle had been identified, small electrode adjustments were made until a site was found in which spontaneous, pulse-synchronous bursts of neural activity could be recorded.

Bursts identified by inspection of the mean voltage neurogram were expressed as burst frequency (bursts per minute), burst incidence (bursts per 100 heartbeats) and median burst amplitude. Median burst amplitude is a sensitive indicator of sympathetic nerve traffic[75].

As MSNA is under the inhibitory control of the arterial baroreflex, the bursts are consequently in cardiac rhythmicity and inversely related to spontaneous blood pressure variations, Figure 4.

Remarks on microneurography

Microneurography is a well validated method to examine sympathetic nerve activity[76]. The method is however time and resource demanding and needs a skilled performer, thus rarely performed in large populations. Microneurography is a more sophisticated method to measure activity in the sympathetic nervous system than an blood sample of noradrenaline[77]. Although MSNA only represents one subdivision of the sympathetic nervous system, it correlates well with global measures of sympathetic nerve activity such as total body noradrenaline spill-over, and with regional (heart and kidney) noradrenaline spill-over[78,79]. MSNA has been shown to have strong intra-individual reproducibility over many years which makes monitoring long-term changes in MSNA possible, both in disease and in therapeutic interventions[80,81].

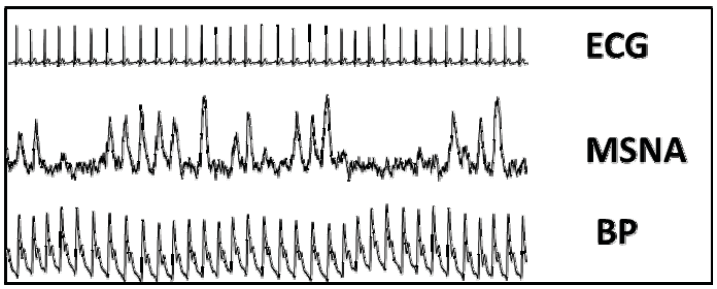


Figure 4. Relation between cardiac rhythmicity, bursts of muscle sympathetic nerve activity and spontaneous blood pressure variations.

During the microneurographic recording in this study, finger arterial blood pressure was measured non-invasively by the volume-clamp method (Finapress 2300, Ohmeda, LA, USA)[82], heart rate was monitored via ECG-chest electrodes and respiration via a strain-gage strapped around the waist.

Stroop color word test

To evaluate the study subjects' cardiovascular response to mental stress, a Stroop color word test (CWT) was performed. The subject was seated in a quiet room with dimmed light for at least ten minutes. Blood pressure and heart rate were recorded after another five to ten minutes of rest, followed by a brief oral instruction to the subject. Oral instructions and measurements of blood pressure and heart rate recordings were done by the same nurse. Blood pressure and heart rate was recorded every other minute; in this study with an automatic Omron 750IT device (Omron Healthcare Co. Ltd, Kyoto, Japan). Blood pressure and heart rate were measured again five and ten minutes after the test was finished. Mean values for blood pressure and heart rate at rest, pre- and post-test, as well as during the stress test, were calculated and used in the analyses.

Remarks on Stroop color word test

Stroop color word test is a video-displayed color word test proceeding for ten minutes during which blood pressure and heart rate is recorded every other minute. Test-retest reliability of CWT has been evaluated[83] and although there is variability in cardiovascular responses between individuals, different stress tests create similar cardiovascular response in normotensive as well as hypertensive individuals[84,85].

Pulse wave velocity, augmentation index and aortic (central) blood pressure

Study subjects were examined in a supine position with legs uncrossed after a ten minute rest in a quiet room. Measurements from the sternal notch to the distal recording site (the femoral artery) were done and brachial blood pressure was measured. Immediately after recording brachial blood pressure, the pulse wave velocity (PWV) was evaluated with applanation tonometry. Artery waveforms were recorded with a high-fidelity micro manometer (SPC-301 Millar Instruments, Houston, TX, USA) in the femoral and carotid arteries. Pressure wave transit time is evaluated as the time difference between the first systolic wave from the heart to the aorta and the reflected pressure wave divided by the length of the aorta, giving the pulse wave velocity. Augmentation index (AIX) is a ratio between the amplitude of the first- and the reflected wave to the pulse pressure. To analyse measured data, SphygmoCor (AtCor Medical, Sydney, Australia) was used.

Remarks on pulse wave velocity, augmentation index and aortic (central) blood pressure

The SphygmoCor device calculates the central blood pressure (Ao SBP and Ao DBP), AIX and pulse wave velocity non-invasively, using a transformation formula that derives the pressure wave in ascending aorta from measurements in the peripheral arteries. The SphygmoCor device has been validated regarding accuracy and reproducibility[86].

Pulse wave velocity increases slowly during the first four to five decades in life, with less steep increase thereafter. Velocity above 12 m/s is considered pathological and is a robust marker of increased arterial stiffness. Augmentation index increases most the first five decades in life, after that the increase is not as steep. Augmentation index reflects both vascular stiffness in the aorta and also the endothelial dependent resistance in the peripheral arteries[87]. Both pulse wave velocity and augmentation index are important methods to evaluate the vascular and endothelial function.

Carotid intima-media thickness

Intima-media thickness was evaluated bilaterally in the carotid arteries with high resolution B-mode ultrasonography using a 7 MHz transducer (Acuson, Siemens, Germany). The subject was lying supine in a quiet room. Both common carotid arteries were examined 20-30 mm proximal to the bifurcation and the intima-media thickness was measured as distance lumen-intima and media-adventitia interfaces. The measurements were done with computerized software developed for this purpose. Average intima-media thickness was calculated from a number of measurements. Intima-media thickness of the far wall was used in the analyses.

Echocardiography

Transthoracic echocardiography was performed with a commercially available echo machine (Vivid 7, General Electric Company, USA). Relative wall thickness (RWT) was calculated with the formula (septal thickness + posterior wall thickness)/left ventricular diastolic diameter and expressed as percent. Left ventricular mass was calculated using the corrected ASE-formula, indexed for body height and expressed as left ventricular mass index (LVMi $g/m^{(2,7)}$). In an apical four-chamber view, left and right atrial borders were manually traced in end-systole. Left atrial size was indexed for body height. Atrial inequality was calculated as left atrial area minus right atrial area. This is an alternative method that adjusts left atrial size to body size[88]. Pulsed wave Doppler tissue imaging was performed in apical views with the sample volume placed at the mitral annulus[89]. Longitudinal annulus velocities were measured in systole (S_m), in early diastole (E_m) and in late diastole (A_m). Measurements were made at four points of the mitral annulus, septal-, lateral-, inferior- and anterior wall and the results were averaged.

In an apical four chamber view, Doppler tissue imaging with high frame rate (about 200 frames/second) was used to register longitudinal systolic strain in the basal septum.

Left ventricular geometry pattern was calculated and considered normal when LVMi was $<45 g/m^{(2,7)}$ and RWT <45%. Concentric remodeling was diagnosed when LVMi $<45 g/m^{(2,7)}$ and RWT ≥45%. Criteria for concentric hypertrophy was LVMi $\geq 45 g/m^{(2,7)}$ and RWT ≥45%. Eccentric hypertrophy was diagnosed as LVMi $\geq 45 g/m^{(2,7)}$ and RWT <45%. Diastolic function was evaluated according to guidelines and categorized as a) normal; b) mild diastolic dysfunction, defined as impaired relaxation without evidence of increased filling pressures; c) moderate diastolic dysfunction, defined as impaired relaxation associated with moderate elevation of filling pressures or pseudo-

normal filling, and d) severe diastolic dysfunction, defined as advanced reduction in compliance or reversible or fixed restrictive filling[89].

Transmitral flow was analyzed and the early (E) diastole and atrial (A) velocity were measured. The deceleration time was measured as the interval from the E-wave peak to the decline of velocity to baseline. The ratio between early transmitral flow velocity and early longitudinal myocardial septal velocity, the E/E_m septal was calculated. When E/A ratio and deceleration time were analysed in the different study groups, subjects with moderate to severe diastolic dysfunction (n=3 in the whole study population) were excluded due to the phenomenon of "pseudo-normalisation" with higher values of E/A and shorter deceleration time in presence of higher filling pressure found in worsening diastolic dysfunction[89].

Remarks on echocardiography
All examinations and measurements were performed according to published guidelines from the American Society of Echocardiography (ASE)[89,90]. Measurements were made offline on Echo Pac (General Electric Company, USA) on three different beats and the results were averaged.

Biochemical assays
Venous blood sampling for laboratory analysis was performed between 7.30 and 10 a.m. after overnight fast with the subject in a relaxed sitting position. Blood was drawn from the antecubital vein. The blood was collected in serum gel (SST) vacutainer tubes and EDTA tubes. They were kept on ice until centrifugation at 4°C and 2000 g for 20 minutes. Plasma (P-) and serum (S-), respectively, were transferred to plastic tubes and stored at –70°C until assay. All biochemical analyses were performed at the accredited laboratory of Clinical Chemistry at Sahlgrenska University Hospital (Swedac 1240) according to the manufacturers' protocol.

P-glucose was analysed with a hexokinase-based photometric method (Modular P, Roche/Hitachi, Germany); P-HbA1c and P-noradrenaline with chromatographic method (Kolon Mono-S; Amersham Pharmacia Biotech/Uppsala, Sweden and high performance liquid chromatography (HPLC) with auto sampler; Chromeleon Chromatography Data System, Dionex, CA, USA).

All other analyses were performed with immuno assays; total and physiological active S-testosterone, S-dehydroepiandosterone sulphate (DHEAS), S-leptin, P-renin (direct measurement), S-aldosterone and N-terminal propeptide of type III collagen (Pro-collagen III) with radioimmunoassay (RIA) (testosterone; Access2, Beckman-Coulter, CA, USA, all others; automatic gamma-counter Wizard 1470, Perkin Elmer, Waltham, MA, USA). N-terminal propeptide of type I collagen (PINP) was analysed with immunoradiometric assay (IRMA). S-cortisol and N-terminal pro B-type natriuretic peptide (NT-proBNP) were analysed with electrochemiluminiscens immunoassay (ECLIA) (Cobas 8000 Roche Diagnostics Scandinavia AB). S-follicle stimulating hormone (FSH) and S-luteinizing hormone (LH) were analysed with chemiluminiscent microparticle immunoassay (CMIA) (Architect™, Abbott Laboratories, IL,

USA), as was sex hormone binding globulin (SHBG) (Abbott *i* System, Abbott Laboratories, IL, USA). Insulin-like growth factor 1(IGF-1) was analysed with immunoenzymometric method with chemiluminiscent measure (IEMA) (Immulite® 2500, DPC*/ Siemens Diagnostic Products Corporation, Los Angeles, CA, USA). S-Apo lipoproteins and S-high sensitive CRP (hsCRP) were analysed with immuno turbidimetric method (Modular P800, Roche/Hitachi, Germany).

Questionnaire

Of the 319 invited women, 208 women did not participate in the clinical examinations. Four were deceased, the remaining 204 were followed-up by a questionnaire regarding their pregnancy during 1969–1973, as well as their present health status and medications. The women were asked to specify whether they had experienced a hypertensive pregnancy or not and if they were diagnosed with any of the following diagnosis when answering the questionnaire; hypertension, diabetes mellitus, myocardial infarction or angina pectoris, stroke or transitory ischemic attack. Thirteen women could not recall their blood pressure during pregnancy.

Perceived stress

Perceived stress was assessed with a questionnaire shown to be associated with cardiovascular disease, both in prospective studies[58,59] as well as in the INTERHEART study[57]. Stress was defined as feeling irritable, filled with anxiety or having trouble sleeping. Participants were asked to report how often they had felt stress using the following response options: (1) never, (2) at some period, (3) at some period during the last five years, (4) at several periods during the last five years, (5) permanent stress during the last year, or (6) permanent stress during the last five years. High level of stress was defined as several periods of stress at home, work or both (response options 4–6). This group was categorized as "high stress" and those reporting no or a few periods of stress the last five years were categorized as "low stress" (response options 1-3).

Statistics

Mainly parametric tests were used to compare means between groups. Parametric tests are usually considered as more powerful than non-parametric tests, but at the same time have more stringent requirements. Parametric tests assume that the groups to be compared are normally distributed and not too small and that the data level is scaling. The choice to perform primarily parametric tests (independent t-test and one-way ANOVA) was based on the compared groups being similar in many aspects and the data being of scale level for most variables. Non-parametric statistics (Chi2, Mann-Whitney-U and Kruskal-Wallis test) were used when comparing categorical variables.

Continuous variables are reported as mean ± 1 standard deviation (SD) and categorical variables as mean (percentages). All *P*-values are two-tailed and $P<0.05$ was regarded as significant.

The statistical analyses were performed with SPSS 12.0.1 and Statistica 7 (StatSoft, Tulsa, OK, USA) in Paper I and with IBM SPSS Statistics 19.0 (IBM, Armonk, NY, USA) in Paper II - IV.

Paper I

Parametric tests were used to compare means between the groups, i.e. a one-way ANOVA was used to compare groups 1-3 and independent t-test was performed comparing women with previous hypertensive pregnancies, i.e. women from group 1 and 2 (n=18) to women with previous normotensive pregnancies, group 3 (n=10).

Correlation analysis (Pearson) was performed to explore the relationship between continuous variables. In a power calculation with burst incidence (BI) as the primary measure, a study sample of 28 individuals was considered adequate.

Paper II

Parametric statistics were chosen due to continuous measures and non-parametric test were conducted when appropriate. Independent samples t-tests were used to compare the HTP group with the NTP group. Chi2 tests were used when exploring categorical variables. Laboratory analysis not normally distributed (hsCRP, HbA1c, leptin, renin, aldosterone, noradrenaline) were log transformed before analysis.

Paper III

Besides comparing women with HTP to women with NTP, the study population was categorized in two groups; high versus low stress and means of continuous variables were compared with parametric tests. Non-parametric statistics were used when appropriate.

A multiple linear regression model was used to assess the ability of diagnosis of hypertension, plasma HbA1c, levels of stress and serum cortisol to predict waist circumference after controlling for age and height. Waist circumference was log transformed to reach normal distribution.

Paper IV

The data was analyzed with respect to previous blood pressure during pregnancy (HTP and NTP respectively), a current diagnosis of hypertension, systolic ambulatory blood pressure above or below the statistical median and duration of hypertension.

Comparisons of means between groups were performed with t-test and with Chi2 test when appropriate. Kruskal-Wallis test was used to compare means between groups regarding duration of hypertension.

RESULTS

Paper I: Sympathetic nerve activity in women 40 years after a hypertensive pregnancy

The main aim of this study was to measure sympathetic nerve activity in women with previous hypertensive pregnancies in comparison to women with normotensive pregnancies. Muscle sympathetic nerve activity (MSNA) was measured with microneurography.

Women with previous hypertensive pregnancies did not - as a group - have enhanced sympathetic activity and MSNA expressed as burst frequency, burst incidence and burst amplitude distribution did not differ when compared to women with normotensive pregnancies (Table 1).

Table 1. Heart rate and sympathetic nerve activity in women with previous hypertensive pregnancies (groups 1+2) vs. normotensive pregnancies (group 3)

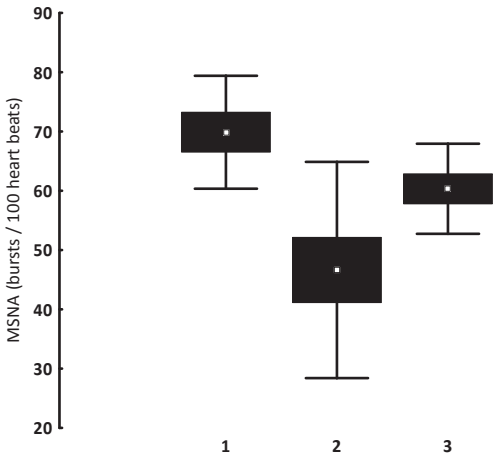
Variable	**Groups 1+2 n = 18**	**Group 3 n= 10**	***P* value**
Heart rate (beats/min)	63 ± 7	62 ± 7	ns
MSNA (BF)	36 ± 12	38 ± 4.6	ns
MSNA (BI)	56 ± 19	61 ± 7.8	ns
MSNA (mamp)	41 ± 8.7	41 ± 4.7	ns
Baroreflex slopes (r-value)	-0.08 ± (-0.16)	-0.17 ± (-0.12)	ns

Group 1: women with previous hypertensive pregnancies and present hypertension, Group 2: women with previous hypertensive pregnancies, now normotensive, Group 3: women with normotensive pregnancies, now normotensive, MSNA: Muscle Sympathetic Nerve Activity, BF: burst frequency, BI: burst incidence, mamp: median burst amplitude, ns: non-significant. Results are presented as the mean ± SD

However, in women with previous hypertensive pregnancies and a current diagnosis of hypertension, MSNA BF and BI were elevated compared to women currently normotensive irrespective of blood pressure status during previous pregnancy (Figure 5).

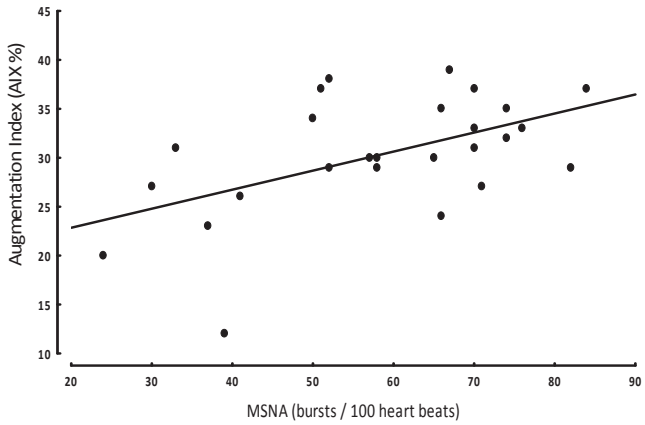
When assessed for the whole study group (n=28) MSNA was positively related to the AIX (r=0.53, *P*=0.006, Figure 6) and systolic blood pressure (r=0.43, *P*=0.02) and inversely related to S-LH (r= -0.5, *P*=0.02).

In women with previous hypertensive pregnancies and still hypertensive (group 1) MSNA was inversely related to DHEAS concentration (r= -0.76, *P*=0.05), but not in the other groups.



Group 1: women with previous hypertensive pregnancies and present hypertension, group 2: women with previous hypertensive pregnancies, now normotensive, group 3: women with normotensive pregnancies, now normotensive. Difference in BI between group 1 *vs*. 3 $P \leq 0.05$, between group 1 *vs.* 2 $P \leq 0.005$

Figure 5. Shows MSNA expressed as burst incidence in the three study groups.



Result from one subject is overlying and result from one subject is missing.
r=0.53, p=0.006

Figure 6. Correlation between MSNA expressed as burst incidence and the AIX for the whole study group.

Baroreflex sensitivity (analysed as relation between MSNA and DBP) to the vasculature was decreased in women with hypertensive pregnancies and current hypertension compared to normotensive women irrespective of previous pregnancy blood pressure status.

Regarding blood pressure measurements women with previous hypertensive pregnancies and now normotensive (group 2), had significantly lower systolic blood pressure compared to the other groups, but no other differences were found regarding office- or ambulatory blood pressures. Results from the Stroop color word test demonstrated that hypertensive women (group 1) had significantly higher systolic and diastolic blood pressure values before start, during and after the stress test compared with women in groups 2 and 3 but the cardiovascular response to stress was however similar between the groups. Heart rate did not differ between the groups before, during or after the color word test.

Measurements of arterial stiffness (pulse wave velocity and augmentation index) and left ventricular mass were numerically highest among women with hypertensive pregnancies and current hypertension (group 1), but the difference did not reach statistical significance compared to the other groups.

Paper II: Cardiovascular and metabolic characteristics after hypertensive pregnancies

In this study we examined the hypothesis that different cardiovascular mechanisms are changed in women who have suffered hypertensive pregnancies. A follow-up questionnaire regarding cardiovascular and metabolic status was assessed in women not taking part in the clinical examinations.

The main findings were a higher pulse wave velocity and higher levels of plasma glucose, HbA1c and noradrenaline in women with previous hypertensive pregnancies compared to women with normotensive pregnancies (Table 2).

Also, women with previous hypertensive pregnancies had higher prevalence of hypertension; 50% compared to 31% of the women with normotensive pregnancies but the groups did not differ in office- or ambulatory blood pressure measurements (Table 2). The groups did not differ in treatment with antihypertensive agents or any other medication.

One hundred-sixty of 204 (78%) women responded to the questionnaire. Among these women 51% self-reported hypertensive pregnancies and 41% normotensive pregnancies. Thirteen (8%) of responders could not recall blood pressure status during pregnancy, thus were not analyzed further. The self-reported prevalence of ischemic heart disease, stroke/TIA and type 2 diabetes mellitus was higher among women who reported previous hypertensive pregnancies (Table 3).

Table 2. Comparisons between women according to blood pressure status during previous pregnancy

n	**HTP 50**	**NTP 55**	**t-test *P*-value**	**Reference values**
Age; years	63 (6)	63 (5)	0.99	
BMI; kg/m^2	28 (5)	26 (5)	0.23	
WC; cm	91 (13)	89 (13)	0.28	
HT; n	25 (50%)	17 (31%)	*0.046*#	
DM; n	3 (6%)	0	0.065#	
SBP; mmHg	144 (18)	141 (20)	0.43	
DBP; mmHg	87 (10)	85 (11)	0.28	
ABPM SBP; mmHg	126 (11)	123 (13)	0.25	
ABPM DBP; mmHg	74 (7)	73 (7)	0.55	
Ao SBP; mmHg	133 (19)	129 (18)	0.29	
Ao DBP; mmHg	85 (10)	82 (10)	027	
PWV; m/s	8.8 (2,6)	7.8 (1,7)	*0.021*	
AIX; %	29 (7)	31 (6)	0.31	
P-glucose; mmol/L	5.7 (1.2)	5.3 (0.6)	*0.022*	4.2 - 6.3
P-HbA1c; %	4.4 (0.5)	4.2 (0.3)	*0.010*	4 - 5.3
P-noradrenaline; nmol/L	2.45 (0.87)	2.11 (0.80)	*0.040*	0.18 - 2.36

HTP: hypertensive pregnancy, NTP: normotensive pregnancy, BMI: body mass index, WC: waist circumference, HT: current diagnosis of hypertension, DM: diabetes mellitus, SBP: systolic blood pressure, DBP: diastolic blood pressure, ABPM: ambulatory blood pressure measurement, Ao SBP: aorta (central) SBP, Ao DBP: aorta (central) DBP, PWV: pulse wave velocity, AIX: augmentation index. #: Pearson chi-2 test. Results presented as mean (SD) for continuous variables and as number (%) for HT and DM

Table 3. Characteristics in women answering questionnaire

Group n	**HTP 81**	**NTP 66**
Hypertension	57 (70)	12 (18)
MI/AP	6 (7)	0
Stroke/TIA	3 (4)	1 (1.5)
DM	16 (20)	2 (3)

HTP: hypertensive pregnancy, NTP: normotensive pregnancy, MI/AP: myocardial infarction/angina pectoris, TIA: transitory ischemic attack, DM: diabetes mellitus. Results presented as numbers (%).

Paper III: Impact of perceived stress on waist circumference in post-menopausal women

Results from studies regarding the possible association between perceived stress and waist circumference have shown centradictory results. With respect to previous hypertensive pregnancies and to a current diagnosis of hypertension, the aims of the present study were to examine the associations between high perceived stress and visceral obesity, metabolic parameters and cardiovascular response to mental stress test. Ninety-six women answered a questionnaire regarding perceived stress of which 43 reported low and 53 high levels of stress respectively. Women reporting high perceived stress were significantly younger than women with low stress and had larger waist circumference despite equal BMI. The prevalence of other cardiovascular risk factors did not differ between the groups. Eighteen women in the low stress group and 29 women in the high stress group had experienced a hypertensive pregnancy (Table 4).

Table 4. Characteristics in relation to perceived stress level

Group **n**	**Low stress** **43**	**High stress** **53**	**t-test** ***P* value**	**Chi-2 test** ***P* value**
BMI; kg/m^2	27 (6)	27 (5)	0.57	
WC; cm	87 (14)	93 (12)	*0.031*	
Age; y	65 (5)	62 (5)	*0.007*	
S-cortisol; nmol/L	403 (157)	387 (129)	0.57	
P-HbA1c; %	4.3 (0.4)	4.4 (0.4)	0.67	
HT; n	17 (40)	24 (45)		0.72
DM; n	0 (-)	3 (6)		0.32
Stroke/TIA; n	2 (5)	2 (4)		1
Smokers; n	7 (16)	9 (17)		1
HTP	18 (42)	29 (55)		0.30
NTP	25 (58)	24 (45)		0.30

BMI: body mass index, WC: waist circumference, HT: current diagnosis of hypertension, DM: current diagnosis of type 2 diabetes mellitus, TIA: transitory ischemic attack, HTP: hypertensive pregnancies, NTP: normotensive pregnancies. Results presented as mean (SD) for continuous variables and as number (%) for HT, DM, stroke/TIA, smokers, HTP and NTP.

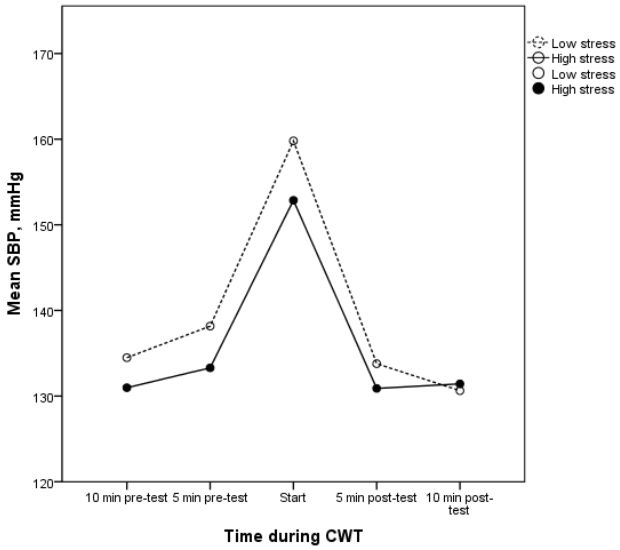
In a multiple regression analysis high levels of stress predicted waist circumference (β=0.22; *P*=0.03), levels of cortisol were inversely (β=-0.25, *P*=0.01) and HbA1c levels positively associated to waist circumference (β=0.22, *P*=0.047). There was no difference in results from the regression analysis whether waist circumference was log transformed or not.

Blood pressure levels, whether recorded as office- or ambulatory blood pressure, metabolic blood profile and serum levels of cortisol did not differ between women with high or low perceived stress. Cardiovascular response to Stroop color word test did not differ at baseline, during or after the test between the groups with respect to perceived levels of stress (Table 5 and Figure 7), neither did cardiovascular reactivity measured as differences in mean blood pressure and heart rate values between pre-stress, stress and post-stress.

Table 5. Blood pressure and heart rate during Stroop color word test in relation to perceived stress level

Group **n**	**Low stress** **43**	**High stress** **53**	**t-test** ***P*-value**
SBP; mmHg			
Pre-stress 5 min	138 (20)	133 (17)	0.21
Start	160 (23)	153 (24)	0.17
Max	171 (23)	168 (24)	0.57
Post-stress 5 min	134 (20)	131 (17)	0.45
DBP; mmHg			
Pre-stress 5 min	85 (10)	82 (10)	0.11
Start	97 (13)	92 (11)	0.062
Max	103 (12)	103 (12)	0.79
Post-stress 5 min	84 (9)	81 (9)	0.18
HR; beats/min			
Pre-stress 5 min	71 (9)	71 (11)	0.79
Start	81 (9)	80 (14)	0.61
Max	86 (9)	84 (14)	0.58
Post-stress 5 min	72 (9)	70 (11)	0.35

SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate. Results presented as mean (SD).



Differences in BP between high and low stress at any time are non-significant, p>0.05.

Figure 7. Systolic blood pressure during Stroop color word test with respect to level of perceived stress.

Paper IV: Echocardiographic changes in relation to blood pressure in postmenopausal women

In this paper, we examined cardiac structure and function in relation to earlier pregnancy blood pressure status and to current blood pressure status and levels. Correlations between early signs of diastolic dysfunction and measurements of vascular stiffening were also investigated.

The main outcomes regarding cardiac variables were found in relation to ambulatory blood pressure levels. Mean systolic ambulatory blood pressure (ABP) in the study population was 124.6 mmHg and the median was 124.0 mmHg. Study subjects with systolic ABP above 124 mmHg had both structural and functional changes compared to the group of women with systolic ABP equal to or below 124 mmHg.

Women with higher systolic ABP had signs of affected diastolic function (lower left ventricular early relaxation, larger left to right atrial inequality and higher E/Em which is a sign of higher left ventricular filling pressure), Table 6.

Table 6. Echocardiographic characteristics, systolic and diastolic function with respect to BP levels above or below median in ABPM

n	**≤ 124 mmHg** **50**	**>124 mmHg** **54**	***P*** **t-test**
RWT; %	40 (5)	43 (6)	*0.043*
LVM; g	120.0 (23.0)	139.5 (32.4)	*0.001*
LVMi; $g/m^{2,7}$	30.9 (5.7)	37.4 (8.9)	*<0.001*
LA size; cm^2	18.0 (2.1)	18.2 (2.5)	0.62
Atrial inequality; cm^2	1.6 (1.2)	2.6 (1.9)	*0.004*
STd; mm	9.1 (1.2)	9.9 (1.3)	*0.005*
PWTd; mm	8.2 (0.9)	8.9 (1.1)	*<0.001*
LVIDd; mm	43.4 (3.5)	44.3 (4.0)	0.20
LVIDs; mm	28.0 (4.1)	29.9 (3.1)	*0.011*
Strain, systolic; %	-18.7 (4.6)	-18.2 (4.0)	0.58
S_m septal; cm/s	6.6 (0.9)	6.6 (1.0)	0.82
E_m septal; cm/s	8.0 (1.6)	6.9 (1.5)	*<0.001*
S_m mean; cm/s	6.9 (1.0)	6.7 (0.9)	0.20
E_m mean; cm/s	8.7 (1.6)	7.4 (1.6)	*<0.001*
A_m mean; cm/s	8.5 (1.5)	8.5 (1.7)	0.93
E/A	1.07 (0.31)	0.91 (0.29)	*0.011*
DT; ms	200 (33)	215 (52)	0.08
E/Em	9.3 (0.02)	11.5 (0.03)	*<0.001*

RWT: relative wall thickness, LVM: left ventricular mass; LVMi: LVM indexed for body mass[2,7], LA size: left atrial antero-posterior size, Atrial inequity: left atrial minus right atrial size, STd: septal thickness diastolic dimension, PWTd: posterior wall thickness diastolic dimension, LVIDd: LV diastolic dimension, LVIDs: LV systolic dimension, S_m: longitudinal annulus velocities in systole, E_m: longitudinal annulus velocities in early diastole, A_m: longitudinal annulus velocities in late diastole, DT: deceleration time. Reference values: longitudinal systolic strain basal septum: -14.6 (3.9)[91]. Results presented as mean (SD).

No differences were found in demographic characteristics or in left ventricular geometry between the groups. Women with systolic ABP above median had higher serum levels of NT-proBNP (127.0 versus 77.0; *P*=0.005), but other laboratory measures did not differ. Previous hypertensive pregnancies per se did not have any influence on cardiac structure or function or any of the other measured variables. A current diagnosis of hypertension as well as a longer duration of hypertension was related to minor but significant signs of impairment in the heart.

We did not find any significant correlation (Pearson´s r= -0.17, *P*=0.09) between myocardial longitudinal velocity measured as septal E max and augmentation index (Figure 8) or between myocardial longitudinal velocity and pulse wave velocity. There was though a significant inverse correlation between myocardial longitudinal velocity and systolic ABP for the whole study population (Pearson r=0.4, *P*<0.001, Figure 9) and between myocardial velocity and serum levels of NT-proBNP (Pearson r=0.31, *P*=0.02).

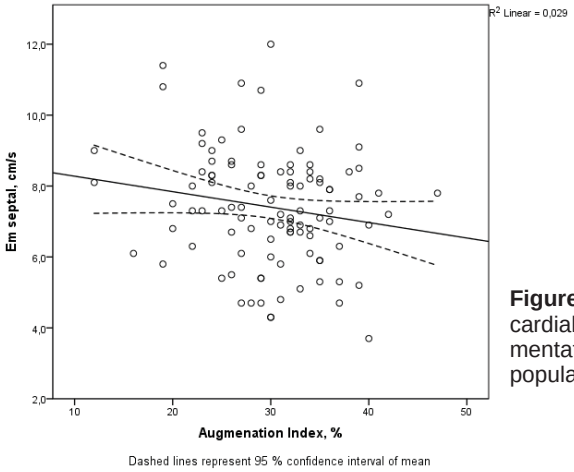


Figure 8. Correlation between myocardial longitudinal velocity and augmentation index in the whole study population.

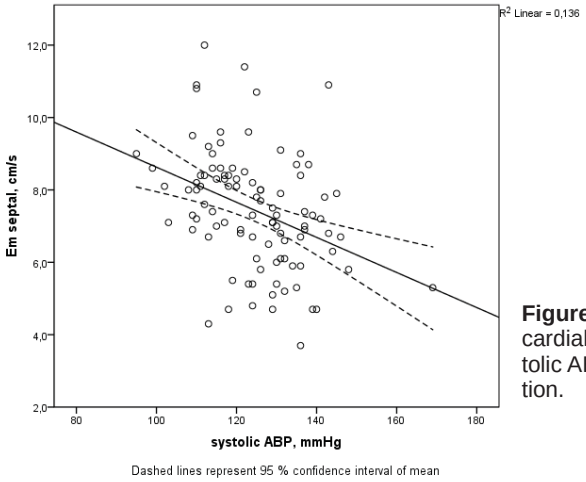


Figure 9. Correlation between myocardial longitudinal velocity and systolic ABP in the whole study population.

DISCUSSION

Findings in hypertensive and normotensive pregnancies

Hypertension

Hypertensive pregnancies are associated with increased prevalence of hypertension later in life. This has been shown in numerous studies[3,4,7] and the pattern is similar in our investigations where 25 of 50 women with previous hypertensive pregnancies had a current diagnosis of hypertension. The same was true in 17 of 55 women with normotensive pregnancies.

Despite a higher prevalence of a diagnosis of hypertension in our study, the blood pressure levels did not differ much between subjects in the different groups. Women were categorized as having hypertension based on their clinical history and thus diagnosed before entering the study. The categorization of the study subjects in hypertensive and normotensive individuals based on history resulted in two populations that were similar regarding current blood pressure levels. This may make it difficult to find differences between the groups when making comparisons with respect to previous as well as to current blood status.

Women with previous hypertensive pregnancies and categorized as normotensive (n=25) did not differ in any examined parameter compared to normotensive women with normotensive pregnancies (n=38). These results speak against major influence of the hypertensive pregnancy per se for future cardiovascular risk. Women who remain normotensive after a hypertensive pregnancy are thus not predetermined to have affected cardiovascular systems many years postpartum. Instead, a diagnosis of hypertension had negative influence on the examined variables. These findings has important clinical implications since it – in some ways – is possible to influence the cardiovascular risk associated with the diagnosis hypertension by refraining from overweight and inactivity.

Sympathetic nerve activity

There is an association between a more permanently increased sympathetic activity and many hypertensive conditions, including pregnancies complicated by hypertension. Other cardiovascular, metabolic and hormonal disturbances such as heart failure, obesity, diabetes mellitus and some endocrine disturbances also exhibit increased sympathetic outflow[26,27,92-94]. When examining the sympathetic nerve activity with microneurography we did not find an impact on the sympathetic system from hypertensive pregnancies per se. Sympathetic activity is more increased during hypertensive compared to normotensive pregnancies[20] and our results indicate that this pathological over-activity is not permanent many years after pregnancy.

In Paper II, women with hypertensive pregnancies had elevated levels of noradrenaline compared to women with normotensive pregnancies. Noradrenaline levels is a more "blunt" measure of the sympathetic system than microneurography, and increased lev-

els can reflect an activated system due to presence of one cardiovascular disease such as hypertension, or to co-existence of more than one cardiovascular risk factor – e.g. hypertension and obesity. This finding of an increased level of noradrenaline may be explained by the higher prevalence of hypertension in the group of women with previous hypertensive pregnancies.

With increasing age, the sympathetic activity escalate and women tend to have a more pronounced increase throughout the years compared to age-matched men[95]. This might be part of the explanation why female sex loses its protective function against cardiovascular disease in older age. Women in Paper I who had enhanced sympathetic activity had a diagnosis of hypertension and were treated with antihypertensive agents when examined. The raised sympathetic outflow that was identified might be a contributing factor to the increased cardiovascular risk seen in individuals with treated hypertension[96].

Baroreceptors in the carotid arteries, aorta and the atriums are important in regulating the sympathetic outflow from the central nervous system. The baroreflex sensitivity and function can be evaluated by analysing the relation between MSNA and diastolic blood pressure. Impaired baroreflex sensitivity seems to be an underlying cause to the increase sympathetic outflow found in the hypertensive women in our study, a result in line with findings from other studies[97-99].

Hormones and blood parameters associated with cardiovascular disease

We could not find any correlation between levels of sex hormones and increased sympathetic activity that could explain a physiological interaction between sex hormones and sympathetic outflow. Instead there was an inverse relation between serum levels of luteinizing hormone (LH) and MSNA, a finding that might be due to chance because of the small number of subjects in the study. Regarding serum levels of dehydroepiandosterone sulphate (DHEAS) we found an inverse relation to MSNA in the group with current hypertension. This could point in the direction of an interaction with the sympathetic system and may be relevant since DHEAS levels decrease with increasing age. This finding is thus physiological explainable, but the small number of examined women must again be underlined. Further the effects of DHEAS on the cardiovascular system are unclear with conflicting results from studies regarding morbidity[100,101]. The other steroid hormones that were analysed did not differ with respect to previous blood pressure status during pregnancy.

Affected metabolic parameters, such as elevated levels of serum insulin, has been shown in follow-up studies after hypertensive pregnancies[102]. We found elevated levels of HbA1c and ApoB/A1 ratio in women with previous hypertensive pregnancies compared to women with normotensive pregnancies, but it seem as if the hypertension diagnosis has influence on these results. When examining all women with a current diagnosis of hypertension, women with previous hypertensive pregnancies (n=25) did not differ in any metabolic parameters compared to women with normotensive pregnancies (n=17) and the possible impact of the hypertensive pregnancy per se seems minor compared to the impact from a diagnosis hypertension.

Other blood parameters interesting for cardiovascular disease such as hsCRP, renin, aldosterone, markers of fibrosis and cardiac peptides were not affected by previous hypertensive pregnancies.

Vascular and cardiac changes

When exploring signs of target organ damage, namely pulse wave velocity, augmentation index, cardiac structure and function and intima-media thickness in the carotid arteries, women with previous hypertensive pregnancies had increased pulse wave velocity compared to women with normotensive pregnancies. Cardiac measures and intima-media thickness were not affected by hypertensive pregnancies in the study population.

A possible contributing factor to the finding of increased pulse wave velocity is - again - the higher prevalence of hypertension after hypertensive pregnancies since hypertension is related to increased arterial stiffness[103]. Hypertension is also associated to cardiac impairment[104]. Whether hypertensive pregnancies per se contribute to arterial stiffness by mechanisms beyond hypertension is not known. In Paper II, when comparing women with a current diagnosis of hypertension with respect to blood pressure status during previous pregnancy, women with hypertensive pregnancies and current hypertension (n=25) had increased pulse wave velocity compared to hypertensive women with former normotensive pregnancies (n=17). These results points toward a possible negative influence of the hypertensive pregnancy by mechanisms not examined in this thesis. In Paper IV we did not report on the plausible differences between women with a current diagnosis of hypertension and previous hypertensive or normotensive pregnancies regarding cardiac variables. However, when comparing the 25 women with previous hypertensive pregnancies and current hypertension to the 17 women with previous normotensive women and current hypertension, no differences were found in cardiac structure or function.

Hence our results imply a negative influence of the hypertensive pregnancies on vascular stiffness but not on cardiac variables. Whether alterations in the vascular bed precede deterioration in the myocardium or if the hypertensive pregnancies have more impact on vascular than cardiac structure is not possible to answer within this study, but it is an interesting question that arises as a result of the present thesis.

Stress

In the INTERHEART study a relation between perceived stress and myocardial infarction was found[57]. The mechanisms are complex and not fully understood, but most likely a number of different processes are involved, including activation of the HPA axis and the sympathetic nervous system. Metabolic disturbances link activation of the "stress systems" to cardiovascular disease. In Paper III we examined the associations between high perceived stress (with the same questions that were used in the INTERHEART study) and visceral obesity and metabolic parameters and between high perceived stress and cardiovascular response to acute mental stress.

If women with hypertensive pregnancies experience more stress than women with normotensive pregnancies and, if so, whether high levels of perceived stress may

contribute to increased cardiovascular risk is not known. This question was answered indirectly in Paper III were women with previous hypertensive pregnancies did not have statistically significant higher levels of perceived stress compared to women with normotensive pregnancies. Neither did they differ in cardiovascular response during mental stress test. To reach a bit closer to an answer whether there is any association between hypertensive pregnancies and perceived stress and cardiovascular response to stress, many more studies with prospective as well as long follow-up designs are needed.

Irrespective of previous pregnancy blood pressure, we found a correlation between high perceived stress and increased waist circumference. The difference was significant despite similar BMI and blood pressure values and women in the "high stress" group did not differ in other metabolic variables compared to women reporting "low stress". A possible explanatory mechanism between perceived stress and visceral obesity is through a more permanently activated HPA axis and the subsequent rise in total serum cortisol. Cortisol receptors are frequent in visceral adiposity tissue and high levels may increase adiposity tissue with a concomitant increase in waist circumference[63].

Women in our study did not differ in morning cortisol levels with respect to perceived stress. It could be expected that women with high perceived stress would have higher levels of cortisol, but lack of difference between the groups can be explained by a more permanent stress which is assessed with the questionnaire used in the study. More permanent stress leads to a constantly activated HPA axis with subsequent altered pattern in cortisol excretion, including a higher total level of serum cortisol but yet lower morning values. A limitation when analysing morning cortisol levels is its normal diurnal variation, thus analyses of a single morning value of cortisol only gives a momentary insight to the complex HPA axis[63].

The blood pressure and heart rate response during the mental stress test were equal between the two groups with respect to perceived stress. Since the cardiovascular response during mental stress test is mainly mediated through the sympathetic nervous system, the results point against major influence from the sympathetic nervous system regarding the impact on waist circumference.

Questionnaire

To compare women who took active part in the clinical follow-up studies with those who chose not to accept the invitation to participate, a questionnaire regarding past and present health was sent by mail to 204 women. One hundred-sixty women answered, giving a response frequency of almost 80%. Of the 147 women who answered the follow-up questionnaire and could recall their blood pressure status during pregnancy, 81 reported previous hypertensive pregnancies. Hypertensive pregnancies had thus the same prevalence as in the whole study population. The same group had higher self-reported prevalence of present hypertension and other cardiovascular diseases. The diagnosis´ have not been verified in medical journals or registers, but still gives a picture of these women´s health. The results from the questionnaire are similar to findings in a recent study showing an increased self-reported cardiovascular morbidity in

women who have experienced hypertensive pregnancies[105]. The clinically examined women in our study hence seem to represent a healthy sub-population of women with previous hypertensive pregnancies, i.e. there is a risk of underestimating cardiovascular changes long time after hypertensive pregnancies when interpreting the results. Selection bias of healthier population to studies is an unfortunate but quite common problem[106, 107].

To summarize, women with hypertensive pregnancies had a higher prevalence of a diagnosis of hypertension and we found some impact on vascular stiffness and metabolic parameters in these women. We did not find any major influence of hypertensive pregnancies on sympathetic activity, on cardiac structure and function, on cardiovascular response to acute mental stress or on levels of perceived stress. Neither was blood parameters associated with inflammation, fibrosis or the renin-angiotensin-aldosterone system affected. Results from the questionnaire showed an increased self-reported cardiovascular morbidity in women with previous hypertensive pregnancies.

Impact of hypertension

Besides investigating the possible long-term impact of hypertensive pregnancies on different outcome variables, we also wanted to explore whether a diagnosis of hypertension was of importance for changes in systems associated to cardiovascular, metabolic and neurohumoral regulation. This approach is reasonable considering the high prevalence of hypertension following hypertensive pregnancies.

Women with a diagnosis of hypertension did not differ much regarding office blood pressure levels compared to women categorized as the normotensive group. When blood pressure was measured with ambulatory readings or as central blood pressure the levels were higher in women with hypertension. Yet, in most women with hypertension the blood pressure levels were within normal range.

In Paper I sympathetic activity was increased in women with a diagnosis of hypertension. However, the same group also had previous hypertensive pregnancies but taken together with the other results, a diagnosis of hypertension seems important regarding our examined outcome variables. For example, pulse wave velocity and augmentation index were higher in numerical values in hypertensive women in Paper I and there was a positive correlation between systolic blood pressure and MSNA. In Paper IV, women with hypertension had higher BMI and larger waist circumference than women without a hypertension diagnosis, and echocardiographic variables - both regarding structure as well as function - were affected in hypertensive compared to normotensive women. Since hypertensive study subjects thus had signs of affected cardiovascular and metabolic parameters the findings point towards a negative impact of a diagnosis of hypertension even though the blood pressure levels were normal or only slightly elevated. The normal or near normal blood pressure levels seemingly reflect that women with a diagnosis of hypertension were well-controlled with antihypertensive treatment (life-style and/or medications). Nevertheless, being diagnosed with hypertension often means years of undiscovered elevated pressure that has affected the vasculature, the heart and other systems in a negative way.

The average duration of a hypertension diagnosis among our study population was 15 years which is the time the individual woman has been aware of her blood pressure elevation. It is not possible to know the "real" duration that the cardiovascular systems have been exposed to high pressures with concomitant wearing. In Paper IV the duration of hypertension had an influence on both structural and functional echocardiographic variables. The negative effects of blood pressure elevation get worse the longer the individual person has been diagnosed with hypertension. Longer duration means longer periods of higher pressure levels increasing target organ damage and consequently increasing the risk for manifest cardiovascular disease.

Importance of blood pressure level

The importance of well controlled blood pressure to decrease cardiovascular risk is underlined by results from our studies. As shown in Paper IV, elevation in blood pressure above a level usually considered as normal, has a negative impact on cardiac structure and function. Ambulatory blood pressure measurements are more closely correlated to cardiovascular morbidity and mortality compared to conventional measurements[108] and our results from Paper IV shows that minor elevation in blood pressure is related to deterioration in the heart.

We chose to compare groups above and below the median systolic ambulatory pressure (124 mmHg) as one way of comparing higher and lower blood pressure levels. Another way to compare groups in relation to blood pressure levels could be to examine systolic levels above and below 135 mmHg. This is the level often used to categorize individuals as hypertensive when using ambulatory measurements, but would result in a comparison similar to comparing normotension versus hypertension. A third possible way to examine the impact of blood pressure levels is to relate highest versus lowest quartile of systolic ambulatory blood pressure, but this selection would result in very small groups due to the size of the study population.

Given the results from the study, the use of ambulatory blood pressure measurements may thus give a better picture of each individual´s risk for cardiac deterioration compared to the use of conventional blood pressure measurements. Our results do not give an answer to the exact level where negative impact on the heart begins and to be able to draw conclusions about precise blood pressure and decline in target organ structure and function, much larger populations would have to be studied. The inverse correlation between systolic blood pressure level and cardiac tissue velocity found in Paper IV does however point toward a negative impact on cardiac function already at minor blood pressure elevation. Diastolic dysfunction is an early sign of hypertensive heart disease and is correlated to morbidity and mortality[45,48]. It is interesting to notice that in the present population of postmenopausal women with low cardiovascular risk and well controlled blood pressure, we could identify an association between blood pressure levels and minor signs of diastolic dysfunction.

Although we found a correlation between blood pressure and cardiac tissue velocity, no significant correlation was found to signs of arterial stiffness. Other studies have found correlations between arterial stiffness and cardiac variables[56,109]. The conflict-

ing results to other studies might be a result of the women in our study being in good health with only a very few affected by clinical cardiovascular manifestations or diabetes mellitus. There may of course also be correlations that go undetected in our study because of the small study sample.

To further comprehend the impact of blood pressure levels in addition to the hypertension diagnosis, it would have been interesting to compare women with diagnosed hypertension and a systolic blood pressure above and below the ambulatory median respectively. However, these groups would comprise of few individuals and with comparisons of many cardiac variables there is an obvious risk of getting results difficult to interpret.

Strengths and limitations

The long follow-up time after pregnancy is the main strength of this study. To the best of our knowledge there are no comparable investigations - with clinical investigations - performed. Considering this, the size of the study population is relatively large and gives insight to the cardiovascular health in postmenopausal women. Women who did not participate in the clinical examinations were invited to take part in a questionnaire regarding previous and present cardiovascular health, and the response rate to this questionnaire was almost 80% which may be considered as high. Together, the results from the clinical examinations and answers from the responders to the questionnaire gives a picture of examined and perceived health in 265 women four decades after pregnancy.

The women participating in the study were investigated with a wide variety of methods examining the cardiovascular system, and the methods used are well established and validated. This enables us to evaluate many different aspects of cardiovascular structure and function. The use of multiple methods can also be considered a limitation since multiple comparisons between different groups increase the possibility of finding false positive associations. On the other hand, there is a risk of not identifying true differences due to small numbers of women in each group when separating the sample in multiple subgroups.

Another aspect that may be considered a limitation is that the majority of women participating in the studies were in good health without clinical cardiovascular manifestations. The groups compared regarding previous pregnancy blood pressure status thus turned out similar in many aspects. This could result in a difficulty to recognize true associations between previous hypertensive pregnancy and later cardiovascular deterioration.

We examined women in the study with on-going antihypertensive treatment. This is a possible limitation since the medications may have diminished sympathetic activity and may also have affected other examined variables.

Since preeclampsia and gestational hypertension can be considered as separate pathophysiological conditions, it would have been interesting to examine women with previous preeclampsia and women with gestational hypertension as separate groups. We though chose to keep all women with previous hypertensive pregnancies as one group due to the size of the study population with small subgroups of preeclampsia and gestational hypertension respectively.

Ethical aspects

When inviting individuals to take part in observational studies one ethical aspect to consider is the possibility to identify medical problems not known by the study subjects. The investigators have to be aware of this aspect and must be prepared to handle the potential problems recognized.

Since the same 105 women comprised the study population in Paper I-IV, there is a risk of publishing the same data and results in different papers. It is of importance to be clear and precise and specify whether the same material and/or methods are used multiple times.

CONCLUSION

The impact of previous hypertensive pregnancies on cardiovascular risk is shown in numerous epidemiological studies but the underlying mechanisms are not fully understood. Results from our studies speak against major influence of the hypertensive pregnancy per se on some of the possible contributing mechanisms for this risk increase, namely sympathetic activity, blood pressure levels and deterioration of cardiac structure and function.

However, women with hypertensive pregnancies 35-40 years ago have a higher prevalence of a diagnosis of hypertension and more self-reported cardiovascular morbidity. There are also signs of an effect of the hypertensive pregnancies per se on vascular stiffness and metabolic parameters which seems to be beyond the diagnosis of current hypertension.

An association was found between levels of perceived stress and waist circumference. Women reporting higher levels of perceived stress had increased waist circumference compared to women with low stress despite BMI being equal in the groups. The increase in waist circumference is possibly related to enhanced activity in the HPA axis and may indicate that stress is a risk factor for visceral fat in postmenopausal women.

The importance of rigorously controlled blood pressure is emphasized by the finding of a relation between slightly higher blood pressure levels and negative impact on cardiac structure and function.

Since very long follow-up investigations after hypertensive pregnancies are rare, our studies contribute with unique material and insights to women´s health many years postpartum. Regarded as a group, women with previous hypertensive pregnancies have an increased cardiovascular risk. The presence of a diagnosis of hypertension seems to be of major importance for this risk increase even though the hypertensive pregnancies in this study contributes with a small but significant influence mainly on vascular function. Thus, maintenance of normotension is essential for women with previous hypertension pregnancies in order to retain cardiovascular health after menopause.

POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Det är välkänt att kvinnor som genomgått en graviditet komplicerad av högt blodtryck löper större risk att senare i livet drabbas av hjärtkärlsjuklighet. För att undersöka mekanismer bakom denna riskökning undersökte vi 105 kvinnor 35–40 år efter graviditet. Hälften av kvinnorna hade haft en graviditet med högt blodtryck och den andra hälften en normal graviditet. Alla genomgick följande undersökningar: mätning av blodtryck och puls med olika metoder, ultraljudsundersökning av hjärtat och bedömning av kärlstelhet, blodtrycks- och pulsreaktion vid stressförsök, skattning av upplevd stress, kontroll av vikt och bukomfång samt analys av olika prover för socker-, hormon- och saltbalans. I en mindre andel av studiepopulationen undersöktes aktivitet i det sympatiska nervsystemet. 160 andra kvinnor svarade på ett frågeformulär angående blodtryck under tidigare graviditet och aktuell hälsa.

Resultaten från studien visar att diagnosen hypertoni (högt blodtryck) var vanligare hos kvinnor som haft blodtrycksförhöjning under graviditet jämfört med kvinnor som genomgått normal graviditet. Samma kvinnor uppvisade tecken på ökad kärlstelhet och viss påverkan på sin blodsockerbalans men inte någon tydlig påverkan på struktur eller funktion i hjärtat. Sammantaget kan fynden delvis förklara den ökade risken för framtida hjärtkärlsjukdom. Aktivitet i det sympatiska nervsystemet var ökad hos kvinnor med tidigare blodtrycksförhöjning under graviditet och aktuell hypertonidiagnos

Vi fann också att kvinnor som angav en högre nivå av självupplevd stress de senaste åren hade ett större midjeomfång än kvinnor med mindre upplevd stress trots att kroppsmassan (BMI) var lika. Större midjeomfång är relaterat till en ökad risk för hjärtkärlsjukdom. Ytterligare ett fynd från studien visar att diskret blodtrycksförhöjning var associerad med viss försämring i hjärtats struktur och funktion, vilket understryker betydelsen av noggrann blodtryckskontroll.

Förekomst av hypertonidiagnos fyrtio år efter graviditet verkar vara en starkt bidragande orsak till den välkända ökade risken för hjärtkärlsjukdom efter graviditet med blodtrycksförhöjning För kvinnor med högt blodtryck under graviditet är det viktigt att i möjligaste mån ha ett normalt blodtryck efter graviditet för att bevara ett friskt hjärtkärlsystem efter klimakteriet.

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